Cell, Vol. 80, 7–10, January 13, 1995, Copyright © 1995 by Cell Press

Escape from "Horror Autotoxicus": Pathogenesis and Treatment of Autoimmune Disease

Minireview

Lawrence Steinman
Department of Neurology and Neurological Sciences
Beckman Center for Molecular and Genetic Medicine
Stanford University School of Medicine
Stanford, California 94305-5429

At the beginning of the twentieth century, Ehrlich introduced the concept of "horror autotoxicus." He argued that the immune system was prevented from mounting an attack against the tissues it was designed to protect. At midcentury, Burnet formulated his clonal selection theory, which assumed that self-reactive lymphocytes were eliminated. We now know that autoreactive T and B cells can be readily detected, even in healthy individuals. Potent regulatory mechanisms that keep these cells in check have been discovered. When these controls fail, autoimmune disease ensues. Of adults in Europe and North America, 5% (more than two thirds of them women) suffer from autoimmune diseases. Fortunately, our understanding of the pathogenesis of these diseases is increasing rapidly, and elegant strategies for treatment are being tested in the clinic.

The Natural Occurrence of Autoreactive T Cells: Passing through Thymic Selection

Many, but not all, self-reactive T cells are eliminated by negative selection in the thymus. In the process of negative selection, self-reactive T cells die when they encounter self-antigen in the thymus. However, autoreactive T cells that have escaped thymic selection are not exceptional (Pette et al., 1990). In healthy individuals, self-tolerance is thus maintained in part through mechanisms acting outside the thymus that keep these autoreactive lymphocytes under control. It was thought that self-reactive cells may escape negative selection because the self-antigen is not expressed in the thymus. However, there are two well-characterized circumstances in which self-antigens that are expressed in the thymus are recognized by T cells that migrate to the periphery. Myelin basic protein (MBP) and acetylcholine receptor (AChR) are present in the thymus during development of the immune system (Pribyl et al., 1993), and T cells with specificity for these antigens are detectable in the blood of normal laboratory animals and healthy humans. However, sometimes the mechanisms that keep these autoreactive T cells under control fail. Several factors can contribute to such failure, including the inheritance of certain genetic alleles and exposure to certain pathogens.

Genes Conferring Susceptibility to Autoimmune Disease

Susceptibility to several autoimmune diseases is linked to the major histocompatability compex (MHC) class II HLA genes. Perhaps the two clearest examples of HLA class II associations with disease are seen with insulin-dependent diabetes mellitus (IDDM) and pemphigus vulgaris (PV). In 1987 McDevitt, Todd, and Bell, as well as Ehrlich independently, observed that resistance to IDDM correlates with the presence of the amino acid aspartate at postition 57 of the HLA-DQ molecule. In contrast, HLA-DQβ chains with serine, alanine, or valine at position 57 are associated with an increased risk of IDDM (Nepom and Ehrlich, 1991). In PV, a unique HLA-DQβ allele, differing from closely related alleles at position 57, was found exclusively in patients with PV. The crystal structure of HLA class II illuminates the importance of residue 57, which is situated at one side of the HLA-binding groove. Only certain autoantigens bearing a specific characteristic net charge, appropriate size, and hydrophilicity would be accommodated in the groove when particular amino acids are situated at position 57. Moreover, position 57 is aligned to interact with amino acids in the α chain. The influence of certain DQα and DQβ sequences in combination promotes susceptibility or resistance to IDDM and PV.

Several genes outside the MHC influence the development of autoimmune disease. Susceptibility to IDDM has been linked to at least seven distinct loci in humans (Davies et al., 1994), with MHC class II having the strongest influence. In NOD mice, which develop IDDM spontaneously, susceptibility is associated with several genes, though MHC class II has the strongest linkage. One gene conferring susceptibility to IDDM, named *bcg*, determines resistance or susceptibility to infectious diseases, including Leishmania, Salmonella, and tuberculosis (Cornall et al., 1991). The interaction of disease susceptibility genes and environmental factors is critical in autoimmune disease.

A Role for Microbes in the Autoimmune Response

Microbial superantigens trigger clinical relapses in autoimmune disease. In experimental autoimmune encephalomyelitis (EAE), T cells expressing Vβ8 T cell receptors (TCRs) engage a fragment of MBP, leading to demyelination and paralysis. The bacterial superantigen staphylococcal enterotoxin B activates Vβ8-expressing T cells. It had been assumed that antigens such as MBP were "protected" from the immune system by virtue of their predominant expression in the central nervous system, which is separated in part from the immune system by the blood–brain barrier. However, T cells that are exposed to MBP in the thymus and that are then activated by exogenous factors (like microbial superantigen) traverse the blood–brain barrier and thus may encounter MBP at the site of disease, where they can induce clinical relapses. Microbial activation of autoreactive T cells may be a widespread phenomenon responsible for the relapsing–remitting nature of many autoimmune diseases. Marrack has proposed a mechanism for the pathogenesis of rheumatoid arthritis (RA) in which an unknown superantigen activates disease-mediating T cells and allows these activated cells to enter the synovial tissue, where they persist because of reactivation by autoantigens (Paliard et al., 1991). A superantigen associated with pancreatic islets may be involved in the pathogenesis of IDDM (Conrad et al., 1994).

The significance of infectious pathogens in autoimmunity was demonstrated in two genetically engineered strains of mice. Interleukin-2 (IL-2) knockout mice develop inflammatory bowel disease, resembling ulcerative colitis, though the inflammatory bowel disease is not seen in

Table 1. Self-Antigens That Trigger Autoimmune Disease

Disease	Self-Antigen	Proof
MG	AChR	Conclusive
Graves' disease	TSH receptor	Conclusive
PV	Desmoglein I	Conclusive
Stiff-man syndrome	GAD	Conclusive
LEMS	Synaptotagmin in voltage-gated calcium channels	Conclusive
Isaac's syndrome	Voltage-gated potassium channels	Conclusive
Paraneoplastic encephalitis	RNA-binding protein HuD	Conclusive
MS	MBP	Strong
IDDM	GAD and heat shock protein	Strong
Primary biliary chirrhosis	Dihydrolipoamide acetyltransferase	Strong
Scleroderma	Topoisomerase I	Strong
Uveitis	Interphotoreceptor retinoid-binding protein and S antigen or rod outer segment	Strong

There are three ways to prove conclusively that an immune response to an antigen causes autoimmune disease. First, if removal of the antibody or the T cells reacting to the antigen cures the disease, then it is reasonable to conclude that the antibody or T cells cause the disease. Next, if transfer of either specific antibody or antigen-specific T cells recapitulates the disease in an experimental animal, then again one could conclude that the antibody or T cell is the cause of the autoimmune disease. Finally, in certain autoimmune diseases (including MG, Graves' disease, PV, LEMS, Isaac's syndrome, and stiff-man syndrome), it has been possible to transfer the putative disease-causing antibody from human to experimental animal and to recapitulate the disease.

germ-free conditions (Sadlack et al., 1993). In a second example, in transgenic mice that express a TCR specific for MBP, EAE develops spontaneously, but the incidence is very low when these mice are raised in nonspecific pathogen-free conditions. Infection increases penetrance of the disease (Goverman et al., 1993).

Another way in which pathogens contribute to autoimmunity is through molecular mimicry, which refers to structural homologies between a self-protein and a protein in a viral or bacterial pathogen. Structural similarities between infectious pathogens and self-antigens may lead, in the course of an immune response to a pathogen, to inadvertant autosensitization. For example, MBP shares extensive homologies at the amino acid level with proteins from a number of common pathogens, including measles and hepatitis B. Rabbits developed EAE when immunized with a peptide sequence from hepatitis B with homology to MBP (Fujinami and Oldstone, 1985). Homology may be necessary at only a few of the amino acids comprising a T cell epitope: conservation of the native amino acid sequence at only 4 of 11 amino acids of an MBP epitope is sufficient to induce EAE.

Self-Antigens That Trigger Autoimmune Disease

For most autoimmune diseases, the specific self-antigen that triggers the immune system is unknown. For such conditions as systemic lupus erythematosus (SLE), Sjogren's syndrome, RA, and polymyositis, a strong candidate for a particular antigen involved in pathogenesis has not yet emerged from a wide group of T cell and autoantibody responses that are detected. The few diseases for which we know or suspect the identity of the the self-antigen that elicits the autoimmune response are listed in Table 1.

Three of the diseases for which we know the identity of the self-antigen are autoimmune diseases at the neuromuscular junction. A breakdown in the regulation of T and B cell tolerance to AChR leads to myasthenia gravis (MG). Antibody to AChR causes MG: infants of myasthenic mothers develop transient myasthenia from the transfer of anti-AChR immunoglobulin G (IgG) across the placenta. Plasmapheresis ameliorates the symptoms of myasthenia as the anti-AChR titers are lowered, while thymectomy often cures the disease. Anti-AChR antibodies from humans can transfer the disease to mice. Another autoimmune disease of the neuromuscular junction, the Lambert–Eaton myasthenic syndrome (LEMS), illuminates a fascinating interaction between cancer cells and the the immune system. This disease is characterized by proximal muscle weakness and autonomic dysfunction, including dry mouth and impotence. Most patients with this disease have small-cell lung cancer. LEMS patients make an antibody to an antigen on these cancer cells that is cross-reactive with synaptotagmin, found at neuromuscular junctions. Isaac's syndrome (acquired neuromyotonia) is a third autoimmune disease of the neuromusular junction. Like LEMS, Isaac's syndrome is also associated with cancer, and patients suffer from continuous muscle contraction and excessive sweating. Autoantibodies are directed to voltage-gated potassium channels (Vincent and Newsom-Davis, 1994).

Blister formation of the skin and mucous membranes, which can be life threatening, are the clinical manifestations of PV. PV is mediated by antibodies that interfere with cell-to-cell contact of keratinocytes. The disease can be transferred to experimental animals by the immunoglobulins purified from patients. The autoantibodies in this disease target a keratinocyte surface protein, desmoglein I, a member of the cadherin family of cell adhesion molecules (Amagai et al., 1991).

Multiple sclerosis (MS) is a chronic disease involving an inflammatory reaction within the white matter of the central nervous system mediated by T cells, B cells, and macrophages. The target of the inflammatory response in MS has been elusive. Analysis of the TCR gene rearrangements in the MS lesion has indicated that there are a restricted group of CDR3 motifs (Oksenberg et al., 1993) that are identical to those found in several human and rodent T cell clones reactive to MBP peptides 87–99. The B cell response to MBP in MS has also been studied extensively. Of 116 chronic progressive MS patients, 111 had anti-MBP titers in the cerebrospinal fluid (CSF), while 173 of 180

relapsing MS patients had anti-MBP antibody in the CSF. Most of the patients who had no anti-MBP antibody in the CSF did have antibody to proteolipid protein of myelin. IgG purified from brain lesions reacted with the same region of MBP, p75-106, that is the immunodominant T cell epitope in MS (Warren et al., 1994). Taken together, these data indicate that a cellular and humoral immune response to this region of MBP may be critical in MS. Although antibodies to myelin oligodendroglial glycoprotein and proteolipid protein are also found in the CSF of some MS patients, the major response in 90% of patients is directed to MBP.

In IDDM, 80% of patients have antibodies to GAD_{65} (glutamic acid decarboxylase). GAD_{65} is the initial antigen attacked in NOD mice, and tolerization to this antigen in young mice prevents diabetes (Kaufman et al., 1993; Tisch et al., 1993). Another autoimmune disease, stiff-man syndrome, is associated with an autoimmune response to GAD_{65}. In this disease, stiffness of muscles and painful spasms are related to a defect in the metabolism of the neurotransmitter γ-amino butyric acid. One third of patients with stiff-man syndrome have IDDM as well (Kim et al., 1994).

After an Initial Response to Self, the Autoimmune Repertoire Expands: The Concept of Determinant Spreading

The target of attack remains enigmatic in most autoimmune diseases. One of the problems in identifying the antigens that trigger these diseases is that after the initial autoimmune response, there is a diversification of the T and B cell responses to other antigens at the site of inflammation. This concept has been named "determinant spreading" by Sercarz and colleagues. Determinant spreading was first demonstrated in EAE and then in spontaneous insulin-dependent diabetes in the NOD mouse (Kaufman et al., 1993; Tisch et al., 1993). Determinant spreading reflects a chain of immune responses. Initially, the immune response is directed to a peptide epitope on the inciting antigen. The response then spreads to other peptide epitopes on the same protein (intramolecular spreading) and then to different epitopes on different proteins (intermolecular spreading) within the target tissue.

In the NOD model of IDDM, the T cell response was initially confined to a limited region of GAD. The response then spread to additional determinants of GAD and then to other antigens, including heat shock proteins expressed in the pancreatic β cells. The importance of other antigens, especially HSP60, in the pathogenesis of IDDM has been established. Tolerization to HSP60 leads to reversal of diabetes in NOD mice (Elias and Cohen, 1994).

Both intramolecular and intermolecular determinant spreading has been demonstrated in many other autoimmune diseases. In MG, both the T and B cell responses target multiple regions on AChR, indicating intramolecular spread. Stimulatory autoantibodies directed to certain epitopes on the TSH receptor elicit hyperthyroidism in Graves' disease, while blocking antibodies to other epitopes on the TSH receptor cause primary autoimmune myxedema. In Graves' disease, T cell clones isolated from the inflammatory thyroid infiltrates revealed reactivity to several determinants on thyroid peroxidase, suggesting intermolecular determinant spreading from TSH receptor. In primary biliary cirrhosis, autoantibodies are directed against a wide family of branched chain keto acid dehydrogenase complexes, indicating intermolecular determinant spreading. In scleroderma patients, a pathogenic autoantibody directed to topoisomerase I has been detected that recognizes a cross-reactive epitope in the tight skin mouse (Muryoi et al., 1992). Also seen in scleroderma are autoantibodies against RNA polymerase transcription factors and other targets in the nucleolus organizer region, indicating intermolecular spreading.

T Cells, Cytokines, and Adhesion Molecules in the Regulation of Autoimmunity

Genetic engineering of mice has been useful in elucidating the action of particular genes in the development of autoimmunity. An important role for the CD8 coreceptor on T cells in clinical remission of EAE was demonstrated using $CD8^{-/-}$ mice: relapses of disease were more common for $CD8^{-/-}$ mice than for $CD8^{+/+}$ mice (Koh et al., 1992). That some T cells protect against autoimmunity was also demonstrated by Lafaille and colleagues, who developed transgenic mice with a TCR for MBP and crossed them to RAG1-deficient mice (mice deficient in the ability to rearrange TCR). Only 14% of the TCR transgenic mice who were $RAG1^{+}$ developed EAE, while 100% of the TCR transgenic mice who were $RAG1^{-}$ developed disease. Thus, the nontransgenic lymphocytes protected against disease, and clarification of their identity is underway (Lafaille et al., 1994).

Some cytokines appear to protect against autoimmune disease whereas others promote disease. Transforming growth factor β1 (TGFβ1) knockout mice had inflammatory lesions in heart, liver, and lungs, indicating that TGFβ1 is a critical anti-inflammatory cytokine (Shull et al., 1992). IL-10 knockout mice developed chronic enterocolitis (Kuhn et al., 1993). MRL/lpr and gld mice, defective in fas and fas ligand, respectively, manifest many of the features of SLE in humans, including glomerulonephritis, vasculitis, and a broad array of autoantibodies. Since fas, a member of the tumor necrosis factor (TNF) receptor family, normally transduces an apoptotic signal, these mice demonstrate a connection between aberrantly regulated apoptosis and autoimmune disease (Takahashi et al., 1994). Although the above examples all show that loss of cytokine signals leads to autoimmunity, aberrant cytokine expression can also cause an autoimmune response. For example, transgenic mice expressing TNFα develop spontaneous inflammatory arthritis.

Different types of T cells secrete different types of cytokines, which have different effects on the immune response. Cytokines themselves have been used effectively to treat disease in animal models. IDDM in the NOD mouse, as well as EAE, can be triggered with T lymphocytes bearing a Th1 phenotype, which produces IL-2, interferon-γ, and TNF. Th2 lymphocytes produce IL-10 and IL-4. This has stimulated strategies to treat diseases mediated by Th1 T cells, with cytokines produced by Th2 cells. In NOD mice, IL-4 replacement in vivo prevents IDDM (Rapoport et al., 1993). In EAE, administration of IL-4 prevents the transfer of disease with T cells reactive to MBP. Weiner et al. have isolated T suppressor cells specific for MBP that produce IL-4, IL-10, and TGFβ and that prevent

EAE, even when induced with proteolipid protein of myelin. This nonspecific suppressor mechanism is named innocent bystander suppression (Weiner et al., 1994).

Adhesion molecules have a selective role in the homing of lymphocytes to different anatomic sites. In models of diabetes in the NOD mouse and in EAE, disease can be reversed with antibodies to α4 integrin (Steinman, 1993). While α4 integrin is involved in homing to pancreatic islets and the central nervous system, these molecules are not involved in lymphocyte homing to the inflamed salivary glands seen in the NOD mouse. L-selectin is involved in homing to pancreatic islets, but plays no role in the development of acute EAE lesions. Thus, different adhesion molecules are involved in homing to different target organs, and this specificity may be exploited therapeutically.

Therapeutic Intervention to Restore Self-Tolerance

A number of approaches aimed at restoring self-tolerance in autoimmune disease are now being implemented in clinical trials in humans (reviewed by Steinman, 1993). Some of these trials involve targeting molecules involved in the interaction among T cells, MHC, and self-peptide (Gaur and Fathman, 1994). For example, patients with various autoimmune diseases have been treated with humanized monoclonal antibodies to CD4 and to CDw52. Patients with MS have been immunized against the CDR2 component of a TCR V region recognizing MBP. Relapsing–remitting MS has been successfully treated by blocking antigen interaction with MHC with a copolymer resembling MBP, termed COP1. Attempts to induce tolerance by feeding antigen have given encouraging results in small trials with MS patients and in RA (Weiner et al., 1994).

Interferon-β is now approved by the Food and Drug Administration for clinical use in MS patients for treatment of relapsing–remitting MS. This cytokine may act by down-regulating the expression of interferon-γ and TNF in the MS lesion (reviewed by Steinman, 1993). Blockade of TNFα with a monoclonal antibody has given promising results in trials for treatment of chronic RA and for treatment of exacerbations of inflammatory bowel disease (Elliott et al., 1993). TGFβ trials are in progress for treatment of MS. Other approaches, including blockade of adhesion molecules involved in lymphocyte homing, are underway for treatment of MS and RA. The investment in research on the pathogenesis of autoimmunity is beginning to pay dividends. Prevention and treatments for many of these diseases may be available in the foreseeable future.

References

Amagai, M., Klaus-Kovtun, V., and Stanley, J. (1991). Cell *67*, 869–877.

Conrad, B., Weidmann, E., Trucco, G., Rudert, W. A., Behboo, R., Ricordi, C., Rodriquez-Rilo, H., Finegold, D., and Trucco, M. (1994). Nature *371*, 351–355.

Cornall, R., Prins, J., Todd, J., Pressey, A., Delarato, N., Wicker, L., and Peterson, L. (1991). Nature *353*, 262–265.

Davies, J., Kawaguchi, Y., Bennett, S., Copeman, J., Cordell, H., Pritchard, L., Reed, P., Gough, S., Jenkins, S., Palmer, S., Balfour, K., Rowe, B., Farrall, M., Barnett, A., Bain, S., and Todd, J. (1994). Nature *371*, 130–135.

Elias, D., and Cohen, I. R. (1994). Lancet *343*, 704–706.

Elliott, M., Maini, R., and Feldmann, M. (1993). Arthrit. Rheum. *36*, 1681–1690.

Fujinami, R. S., and Oldstone, M. B. A. (1985). Science *230*, 1043–1045.

Gaur, A., and Fathman, C. G. (1994). Adv. Immunol. *56*, 219–265.

Goverman, J., Woods, A., Larson, L., Weiner, L., Hood, L., and Zaller, D. (1993). Cell *72*, 551–560.

Kaufman, D., Clare-Salzler, M., Tian, J., Forsthuber, T., Ting, G., Robinson, P., Atkinson, M., Sercarz, E., Tobin, A., and Lehmann, P. (1993). Nature *366*, 69–72.

Kim, J., Namchuk, M., Bugawan, T., Fu, Q., Jaffe, M., Shi, Y., Aanstoot, H., Turck, C., Erlich, H., Lennon, V., and Baekkeskov, S. (1994). J. Exp. Med. *180*, 595–606.

Koh, D. R., Fung Leung, W. P., Ho, A., Gray, D., Acha-Orbea, H., and Mak, T. W. (1992). Science *256*, 1210–1213.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W. (1993). Cell *75*, 263–274.

Lafaille, J. J., Nagashima, K., Katsuki, M., and Tonegawa, S. (1994). Cell *78*, 399–408.

Muryoi, T., Kasturi, K., Kafina, M., Cram, D., Harrison, L., Sasaki, T., and Bona, C. (1992). J. Exp. Med. *175*, 1103–1109.

Nepom, G. T., and Ehrlich, H. (1991). Annu. Rev. Immunol. *9*, 493–525.

Oksenberg, J. R., Panzara, M. A., Begovich, A. B., Mitchell, D., Erlich, H. A., Murray, R. S., Shimonkevitz, R., Sherritt, M., Rothbard, J., Bernard, C. C. A., and Steinman, L. (1993). Nature *362*, 68–70.

Paliard, X., West, S. G., Lafferty, J. A., Clements, J. R., Kappler, J. W., Marrack, P., and Kotzin, B. L. (1991). Science *253*, 325–329.

Pette, M., Fujita, K., Kitze, B., Whitaker, J. N., Kappos, A., and Wekerle, H. (1990). Neurology *40*, 1770–1776.

Pribyl, T. M., Campagnoni, C. W., Kampf, K., Kashima, T., Handley, V., McMahon, J., and Campagnoni, A. T. (1993). Proc. Natl. Acad. Sci. USA *90*, 10695–10699.

Rapoport, M., Jaramillo, A., Zipris, D., Lazarus, A., Serreze, D., Leiter, E., Cyopick, P., Danska, J., and Delovitch, T. (1993). J. Exp. Med. *178*, 87–99.

Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A., and Horak, I. (1993). Cell *75*, 253–261.

Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R., Yin, M., Allen, R., Sidman, C., Proetzel, G., and Calvin, D. (1992). Nature *359*, 693–699.

Steinman, L. (1993). Sci. Am. *269*, 106–114.

Takahashi, T., Tanaka, M., Brannan, C. I., Jenkins, N. A., Copeland, N. G., Suda, T., and Nagata, S. (1994). Cell *76*, 969–976.

Tisch, R., Yang, X., Singer, R., Liblau, R., Fugger, L., and McDevitt, H. (1993). Nature *366*, 72–75.

Vincent, A., and Newsom Davis, J. (1994). In Immunology of Neuromuscular Disease, S. R. Hohlfeld, ed. (London: Kluwer), pp. 1–176.

Warren, K. W., Catz, I., Johnson, E., and Mielke, B. (1994). Ann. Neurol. *35*, 280–289.

Weiner, H., Friedman, A., Miller, A., Khoury, S., Al-Sabbagh, A., Santos, L., Sayegh, M., Nussenblatt, R., Trentham, D., and Hafler, D. (1994). Annu. Rev. Immunol. *12*, 809–837.